REMARKS

Claims 1-14, 16-21 and 24-38 are active in this application. Claims 3-14, 16-21 and 24-37 are withdrawn from further consideration as being directed to a non-elected invention. Claims 15, 22 and 23 have previously been cancelled. Claim 38 is newly added. Claims 1 and 2 are examined in this application.

Support for the amendments to claim 1 is found, for example, in original claim 1, in Example 1, and, elsewhere throughout the specification. Claim 1 has been amended to further clarify the invention. Support for the amendments to claim 2 is found, for example, in original claim 2 and elsewhere throughout the specification. Support for new claim 38 is found, for example, in original claim 1, and elsewhere throughout the specification. Applicants submit no new matter has been introduced by this amendment and entry is respectfully requested.

RESTRICTION

At page 2 of the Office Action, the Office has maintained the restriction requirement and made the restriction Final. Applicants have traversed the requirement for restriction and filed a Petition, May 2, 2003 to have the restriction requirement withdrawn. As of the date of this reply, Applicants have not received a decision on the petition. Applicants wish to express their dissatisfaction on the record of having to respond to an Office Action without the benefit of decision on petition filed May 2, 2003.

Applicants thank the Examiner for indicating that claim 1, if limited to SEQ ID NO: 1, would be allowable. In recognition of that indication, Applicants have added new claim 38 directed solely to that embodiment of claim 1. If the petition is granted and the restriction and further requirement for choice of a specific "product" are withdrawn, it is Applicants' understanding

that the Examiner is required to search the other "products" of claim 1, given the indication of allowability of SEQ ID NO: 1.

INFORMATION DISCLOSURE STATEMENT

The Office has returned an executed copy of PTO form 1449 with the Office Action. The 1449 form indicates the Examiner has not considered documents DE 40 03 826 A1 ["DE"] and JP7147986 (sequence listing) ["JP (sequence)"].

DE document

The Examiner notes the DE document has not been considered because an English translation and a statement of the relevance of the DE document to the claimed invention was not provided. Applicants assert the Office has erred in not considering the DE document.

The Search Report for this application was filed at the PTO on August 19, 2000 (see copy of date-stamped receipt post card provided herewith as Attachment A). The family information on the DE document is provided in the Search Report which shows the DE document has a Canadian equivalent published in the English language. The requirement for a concise explanation of relevance can be satisfied by submitting an English-language version of the search report or action which indicates the degree of relevance found by the foreign office. This may be an explanation of which portion of the reference is particularly relevant, to which claims it applies, or merely an "X", "Y", or "A" indication on a search report. Since the Search Report was submitted to the PTO on August 10, 2000, as indicated on the date stamped postcard, Applicants assert the relevance of the DE document has been disclosed to the Examiner (" ...an "A" indication on the Search Report...). Nevertheless, Applicants submit herewith a courtesy of the Canadian equivalent (CA 2,075,366)(Attachment B) of the DE document for consideration by the Examiner. In view of the foregoing, Applicants respectfully request the Examiner to consider the DE document and indicate the consideration on the attached, clean copy of the PTO

1449 previously submitted August 10, 2000 (Attachment C).

JP document (sequence listing)

The Examiner has not considered the JP document (sequence) because "the sequence listing is not provided in a format that would allow the Examiner to ascertain the relevance to the claimed invention." Applicants assert the Office has erred in not considering the JP document (sequence).

Applicants assert they are **not required** to provide a sequence listed in a reference document in CRF format. However, for the convenience of the Examiner, it is noted that the sequence in the JP document (sequence) is publicly available through the NCBI website. Attached hereto is a print-out of the JP sequence obtained through that site (Attachment D). The examiner may access the NCBI website using the following address: http://www.ncbi.nlm.nih.gov. By using the E09420 accession number as listed at the top of the JP (sequence) document and the NCBI website, the Examiner may obtain an electronic copy of the sequence in the JP document in a format suitable for searching. As above, the relevance of the JP document is evidenced by an "A" on the Search Report filed August 10, 2000.

In view of the foregoing, Applicants respectfully request the Examiner consider the JP document (sequence) and to sign, date and initial the clean copy of the August 10, 2000 PTO 1449 form provided herewith.

COMPLIANCE WITH 37 CFR § 1.821-1.825

At page 2 of the Office Action, the Office asserts a computer readable form (CRF) must be submitted in order to comply with the requirements of 37 CFR § 1.821-1.825. Applicants submit herewith a computer readable form (CRF) of the Sequence Listing and a Statement that the content of the paper and computer readable copies are the same. Applicants assert the submission of the CRF and Statement bring this application into compliance with the sequence

rules.

REJECTION UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

At page 3 of the Office Action, the Office rejects claims 1 and 2 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Applicants respectfully traverse the rejection. Claims 1 and 2 are newly amended herein.

The Office asserts the claims are vague and unclear for referencing nonelected subject matter. The Office suggests the claims be amended to reflect the current election (currently SEQ ID NO: 1). In reply thereto, Applicants have added new claim 38 which reflects the amendments to claim 1 suggested by the Examiner. Applicants have not amended claim 1 or 2 as suggested by the Examiner because to do so would moot the pending petition. Moreover, no authority is known, nor has any been cited by the Examiner, to establish the position that failure to restrict to an elected "product" renders an otherwise definite claim vague and unclear.

INDICATION OF ALLOWABLE SUBJECT MATTER

At page 3 of the Office Action, the Office states the erythrovirus genomic segment corresponding to SEQ ID NO: 1 appears to be free of the prior art. Applicants appreciate the notification and have provided allowable new claim 38.

Except for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. § 1.16 and § 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-0310. This paragraph is intended to be a **Constructive Petition for Extension of Time** in accordance with 37 C.F.R. § 1.136(a)(3).

It is believed the application is in condition for examination on the merits and such is respectfully requested. If, in the opinion of the Examiner, an interview would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the telephone number provided below.

Respectfully submitted,
Morgan, Lewis & Bockius LLP

Date: **August 25, 2003**
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Attachments:
Attachment A - Date-stamped post card of August 10, 2000
Attachment B - Canadian Equivalent of DE document (CA 2,075,366)
Attachment C - Clean copy of PTO 1449 form submitted August 10, 2000
Attachment D - Copy of NCBI print-out showing sequence (accession no. E09420)

Amendments to the Claims:

Claims 1-14, 16-21 and 24-38 are pending in the application.

This listing of claims will replace all prior versions and listings of claims in the application.

Please amend the claims as follows:

Claim 1 (currently amended): [A] An isolated nucleic acid comprising nucleic acids selected from the group consisting of:

i) the sequence SEQ ID NO:1,

ii) the genomic sequences of variant erythroviruses, called erythrovirus type V9, which, molecularly, cannot be recognized as an erythrovirus B19 because it exhibits a genetic divergence ≥ 10% over the whole genome with respect to the erythrovirus B19 sequences and which exhibit a genetic divergence of less than or equal to 6% with respect to the sequence SEQ ID NO:1, and

iii) the erythrovirus genomic sequences capable of hybridizing under stringent conditions with one of the following sequences: SEQ ID NO:45-80, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119 and SEQ ID NO:120.

Claim 2 (currently amended): The nucleic [acids] acid of Claim 1 wherein the nucleic [acids] acid exhibits a restriction profile according to Figures 7.1 to 7.3. (SEQ ID NO.: 1)

Claim 3 (withdrawn): Fragments of the nucleic acids according to Claim 1, which are capable of allowing the detection of an erythrovirus V9, characterized in that they are selected from the group consisting of:

a) the sequences SEQ ID NO:81, SEQ ID NO:83, SEQ. ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91 or SEQ ID NO:93,

b) the sequences SEQ ID NO: 2-80

c) the sequences SEQ ID NO:105-121, and

d) the sequences complementary to the preceding sequences, wherein the fragments comprise at least 17 nucleotides derived from the preceding sequences or their complementary sequences.

Claim 4 (withdrawn): A fragment according to Claim 3, selected from the group consisting of the sequences SEQ ID NO:45-80, 108 and NO:110, their complementary sequences, the sequences of at least 17 nucleotides derived from these sequences and the sequences ~~comprising the said sequences and~~ wherein the selected sequence is capable of serving as a probe in the specific identification of an erythrovirus V9 or of a related erythrovirus.

Claim 5 (withdrawn): A fragment according to Claim 3, selected from the group consisting of the sequences SEQ ID NO:2-80 and the sequences SEQ ID NO: 105-121, their complementary sequences, the sequences of at least 17 nucleotides derived from these sequences and the sequences comprising the said sequences and wherein the selected sequence is capable of serving as a primer for the amplification of sequences derived from an erythrovirus.

Claim 6 (withdrawn): A pair of primers, characterized in that they are selected from the group consisting of:

a) pair A: primers SEQ ID NO:111 and SEQ ID NO: 112

b) pair B: primers SEQ ID NO:105 and SEQ ID NO:106;

c) pair C: one of the sequences SEQ ID NO:2-44, 105, 106, 107, 109, 111 or 112 and one of the sequences SEQ ID NO:45-80, 108 or 110;

d) pair D: primer SEQ ID NO:107 and primer SEQ ID NO:109;

pair E: two primers selected from the sequences SEQ ID NO:2-44, 105, 106, 107, 109, 111 or 112; and

pair F: two primers selected from the sequences SEQ ID NO:45-80, 108 or 110.

Claim 7 (withdrawn): A variant erythrovirus, characterized in that it cannot be recognized molecularly as an erythrovirus B19 genome, and in that it exhibits a genetic divergence of less than or equal to 6% with the sequence SEQ ID NO:1 and in that its genome hybridizes specifically, under stringent conditions with one of the sequences SEQ ID NO:45 to 80, 108 and 110.

Claim 8 (withdrawn): A plasmid, characterized in that it comprises the viral genome of a variant erythrovirus strain, called erythrovirus V9, which cannot be recognized molecularly as an erythrovirus B19 and which exhibits with the latter a genetic divergence of $\geq$ 10% over the whole genome with respect to the erythrovirus B19 sequences and a genetic divergence of less than or equal to 6% with the sequence SEQ ID NO:1 or a fragment thereof, according to Claim 3.

Claim 9 (withdrawn): A plasmid, according to Claim 8, characterized in that it includes the sequence SEQ ID NO:1.

Claim 10 (withdrawn): A diagnostic reagent for the differential detection of type V9 erythroviruses, characterized in that it is selected from the sequences SEQ ID NO:45-80, 108 and 110, their complementary sequences, and the sequences of at least 17 nucleotides, derived from these sequences.

Claim 11 (withdrawn): A method for the rapid and differential diagnosis of erythroviruses, by hybridization and/or gene amplification, using a biological sample as starting material, which process is characterized in that it comprises:

(1) a step in which a biological sample to be analyzed is brought into contact with at least one probe of sequence SEQ ID NO:45-80, 108 or 110, and

(2) a step in which the product (s) resulting from the erythrovirus nucleotide sequence-probe interaction is (are) detected by any appropriate means.

Claim 12 (withdrawn): The method according to Claim 11, characterized in that it comprises, prior to step (1):

a step of extracting of the nucleic acid to be detected, belonging to the virus genome, which may be present in the biological sample, and

at least one gene amplification cycle.

Claim 13 (withdrawn): The method according to Claim 12, characterized in that the amplification cycles are carried out with the air of a pair of primers selected from the group consisting of:

i) pair A: primers SEQ ID NO:111 and SEQ ID NO:112;

ii) pair B: primers SEQ ID NO:105 and SEQ ID NO:106;

iii) pair C: one of the sequences SEQ ID NO:2-44, 105, 106, 107, 109, 111 or 112 and one of the sequences SEQ ID NO:45-80, 108 or 110;

iv) pair D: primer SEQ ID NO:107 and primer SEQ ID NO:109;

v) pair E: two primers selected from the sequences SEQ ID NO:2-44, 105, 106, 107, 109, 111 or 112; and

vi) pair F: two primers selected from the sequences SEQ ID NO:45-80, 108 or 110.

Claim 14 (withdrawn): A method for the rapid and differential diagnosis of erythroviruses, characterized in that it comprises:

i) a step of extracting of the nucleic acid to be detected, belonging to the virus genome, which may be present in the biological sample,

at least one gene amplification cycle with the aid of a pair of primers according to Claim 6, and

the detection of the amplified product, on the one hand, by hybridization with the sequence SEQ ID NO:121 and, on the other hand, by the action of the restriction enzyme MunI.

Claim 15 (canceled)

Claim 16 (withdrawn): A method of screening and typing an erythrovirus V9 or a related virus, characterized in that it comprises bringing a probe selected from the group consisting of the sequences according to Claim 4, into contact with the nucleic acid of the virus to be typed, and detecting the nucleic acid-probe hybrid obtained.

Claim 17 (withdrawn): A product of translation, characterized in that it is encoded by a nucleotide sequence according to Claim 1.

Claim 18 (withdrawn): A protein, characterized in that it is capable of being expressed with the aid of a nucleotide sequence according to Claim 1.

Claim 19 (withdrawn): A protein or peptide derived from a variant erythrovirus type V9, as defined in Claim 1 and selected from the sequences:

a) SEQ ID NO:82 (NS1 protein), SEQ ID NO:86 (VP1 protein), SEQ ID NO:88 (single VP1 protein), SEQ ID NO:92 (VP2 protein) and SEQ ID NO:95-104, namely fragments of the VP1 protein [VP1a peptide (SEQ ID NO:95); VP1b peptide (SEQ ID NO:96); VP1c peptide (SEQ ID NO:97); peptide VP1d (SEQ ID NO:98); peptide VP1e (SEQ ID NO:99); peptide VP1f (SEQ ID NO:100)], or fragments of the VP2 protein [peptide VP2a (SEQ ID NO:101); peptide VP2b (SEQ ID NO:102); peptide VP2c (SEQ ID NO:103); peptide VP2d (SEQ ID NO:104)], and

b) the sequences derived from sequences a) comprising between 7 and 50 amino acids.

Claim 20 (withdrawn): An immunogenic composition comprising one or more products of translation of the nucleotide sequences according to Claim 17.

Claim 21 (withdrawn): An antibody directed against one or more of the peptides or proteins according to Claim 17.

Claim 22 (canceled)

Claim 23 (canceled)

Claim 24 (withdrawn): A method of *in vitro* screening diagnosis of infection of an individual with an erythrovirus comprising detecting hybridization of the individual's nucleic acid with a nucleic acid according to Claim 1.

Claim 25 (withdrawn): The method of claim 24 comprising gene amplification.

Claim 26 (withdrawn): The method of claim 16 wherein the probe is labeled.

Claim 27 (withdrawn): The method of claim 16 wherein the nucleic acid of the virus to be typed is labeled.

Claim 28 (withdrawn): An immunogenic composition comprising one or more of the proteins of claim 18.

Claim 29 (withdrawn): An immunogenic composition comprising one or more of the peptides or proteins of claim 19.

Claim 30 (withdrawn): An antibody directed against one or more of the proteins of claim 18.

Claim 31 (withdrawn): An antibody directed against one or more of the proteins of claim 19.

Claim 32 (withdrawn): A method for the immunological *in vitro* screening diagnosis of infection of an individual with an erythrovirus comprising detecting anti-erythrovirus V9 antibodies by contacting a biological sample with a peptide according to claim 17 and detecting the association of such a peptide with antibodies contained in the biological sample by an appropriate means.

Claim 33 (withdrawn): The method of claim 32 wherein the appropriate detection means is selected from the group consisting of EIA, ELISA, RIA, and fluorescence.

Claim 34 (withdrawn): A method for the immunological *in vitro* screening diagnosis of infection of an individual with an erythrovirus comprising detecting erythrovirus V9 viral proteins by contacting a biological sample with an antibody according to claim 21 and detecting the association of such an antibody with erythrovirus V9 viral proteins contained in the biological sample by an appropriate means.

Claim 35 (withdrawn): The method of claim 34 wherein the appropriate detection means is selected from the group consisting of EIA, ELISA, RIA, and fluorescence.

Claim 36 (withdrawn): An erythrovirus diagnostic kit comprising at least one probe of sequence SEQ ID NO: 45-80, 108 or 110, and/or a pair of primers selected from the group consisting of, and/or a peptide encoded by, or capable of being expressed with the aid of a nucleic acid of Claim 1, and/or an antibody directed against such peptides.

Claim 37 (withdrawn): The diagnostic reagent of claim 10 wherein the reagent is labeled with an appropriate marker.

Claim 38 (new): The nucleic acid of claim 1 comprising SEQ ID NO:1.

B' cancld.

NCBI Entrez Nucleotide

PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM Boo

Search Nucleotide for Go Clear

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  AUTHORS   Yamazaki,O., Matsunaga,Y., Takeda,N., Matsuura,Z., Ogawa,H.,
            Shimizu,H., Kamata,K. and Kurosawa,D.
  TITLE     HUMAN PARVOVIRUS GENE, POLYPEPTIDE CODED WITH THE SAME AND USE
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#17

PATENT
ATTORNEY DOCKET NO. 45636-5033-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Quang Tri NGUYEN *et al.*	)	
	)	
Application No.: 09/555,640	)	Group Art Unit: 1648
	)	
Filed: August 10, 2000	)	Examiner: Jeffrey S. Parkin, Ph.D.
	)	
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The undersigned hereby states upon information and belief that the Computer Readable Format (CRF) submitted herewith does not include matter which goes beyond the content of the application as filed and that the information recorded on the diskette submitted herewith is identical to the written Sequence Listing filed June 2, 2000. The CRF and Statement are submitted in response to the Office Action dated February 25, 2003.

Respectfully submitted,
MORGAN, LEWIS & BOCKIUS LLP

Dated: August 25, 2003

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FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Sub Class	Translation YES	Translation NO
		DE 40 03 826 A1	08/14/1991	Germany				X
		WO 96/09391	03/28/1996	WIPO				
		JP 7147986	10/08/1997	Japan (abstract)				
		JP 7147986	06/13/1995	Japan (Sequence Listing)				

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

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(54) Titre : PEPTIDES OU POLYPEPTIDES IMMUNOLOGIQUEMENT ACTIFS TIRES DU PARVOVIRUS B19
(54) Title: IMMUNOLOGICALLY ACTIVE PEPTIDES OR POLYPEPTIDES FROM THE PARVOVIRUS B19

(57) Abrégé/Abstract:
Immunologically active peptides or polypeptides with a partial amino-acid sequence of the capsid proteins VP1 and VP2 of parvovirus B19 which permit tests to be carried out at low cost, sensitively and specifically for the determination of antibodies against human parvovirus B19 are made available. Short peptide sequences which, employed as antigen, serve to identify anti-B19 IgG-positive sera are identified. Furthermore, the production of these peptides using genetic engineering measures is disclosed. Other antigens which are produced by genetic engineering and which can be stably produced in a high yield in E.coli and subsequently purified therefrom are used as additional antigens for IgG detection. Finally, a set of antigens permits tests to be carried out to determine IgM antibodies against the virus. In addition, the components, produced by genetic engineering, of the surface proteins represent substances which can be used for prophylactic immunisation.

ABSTRACT

Immunologically active peptides or polypeptides with a partial amino-acid sequence of the capsid proteins VP1 and VP2 of parvovirus B19 which permit tests to be carried out at low cost, sensitively and specifically for the determination of antibodies against human parvovirus B19 are made available. Short peptide sequences which, employed as antigen, serve to identify anti-B19 IgG-positive sera are identified. Furthermore, the production of these peptides using genetic engineering measures is disclosed. Other antigens which are produced by genetic engineering and which can be stably produced in a high yield in E.coli and subsequently purified therefrom are used as additional antigens for IgG detection. Finally, a set of antigens permits tests to be carried out to determine IgM antibodies against the virus.

In addition, the components, produced by genetic engineering, of the surface proteins represent substances which can be used for prophylactic immunisation.

The human parvovirus B19 (for short hereinafter: B19) was discovered by chance in 1975 in plasma samples from blood donors (Cossart, Y.E., Field, A.M., Cant, B., Widdows, D.: Parvovirus-like particles in human sera. Lancet I (1975) 72-73) by countercurrent electrophoresis. In recent years it has been shown that B19 may cause an aplastic crisis in patients with chronic haemolytic anaemia, and is the aetiological agent of erythema infectiosum (EI).

Under the electron microscope, B19 has a size of about 20 nm. The particles have an icosahedric symmetry. Besides the virus particles there are also seen to be "empty" capsids which contain no DNA. The density in $CsCl_2$ (sic) is 1.36 - 1.40 g/ml. The virus genome consists of a single-stranded DNA of 5.4kb. The nucleotide sequence of the genome of a B19 parvovirus has been derived from a clone which contained virtually the complete viral genome (R.O. Shade et al. Journal of Virology (1986) p. 921). In each case only one DNA strand, either of the plus or the minus orientation, is packaged into each virus particle. B19 is an autonomous parvovirus, that is to say requires no helper virus for replication.

The capsid consists of two polypeptides with molecular weights of 83kDa (VP1) and 58kDa (VP2). In addition, three non-structural proteins of 52, 63 and 71kDa can be detected.

The DNA codes in the 5′ region for the structural proteins of the capsid. The coding regions of the structural proteins are identical apart from an additional N terminus of VP1. This difference is caused by splicing processes at the mRNA level, in which in the case of VP2 the translational start for VP1 is taken out and thus translation can start only with the shorter VP2.

Investigations on various B19 isolates found world-wide have shown that these differ in part at the DNA level by the restriction enzyme pattern. These differences do not, however, correlate with the clinical spectrum of B19 infection.

It has not been possible to date to find a permanent cell line in which B19 can be grown. There has been just as little success to date in establishing an experimental animal model for B19. B19 can, however, be grown in primary bone marrow cells in the presence of erythropoietin. It has thus been possible to clarify the mechanism of replication of the virus and show that cells of erythropoiesis are the target cells of this infection. Inoculation of B19 cells in fetal erythropoietic cells and erythroblasts of a patient with chronic myeloid leukaemia has now succeeded.

B19 causes erythema infectiosum (infectious erythema) which is an infectious disease which usually has a benign course and mostly occurs between the ages of childhood and early adulthood. B19 infection may in addition cause aplastic crises in patients with chronic haemolytic anaemia (sickle cell anaemia etc.) and chronic bone marrow aplasias in patients with inborn or acquired immunodefficiency states.

In pregnancy B19 infection may in about 10-15% result in hydrops fetalis with resulting interuterine death. Furthermore, B19 is associated with the occurrence of Schönlein-Henoch purpura.

As a rule, B19 is transmitted by droplet infection but also by antigen-positive conserved blood and coagulation products.

Since no permanent cell line in which B19 can be obtained in large amounts is yet known, there is thus a lack of a source for obtaining antigen for diagnostic tests. To date one has made do with B19 virus discovered by chance in conserved blood from donors who are just in the viraemic stage of infection.

The object of the present invention is to provide immunologically active polypeptides which permit, with the test systems presented here, detection of B19-specific antibodies of the IgG and IgM class. This results in the following possible applications:

- Serodiagnosis of acute or previous B19 infections in dermatology, haematology, gynaecology, rheumatology and paediatrics.
- Determination of the B19 immune status in pregnant women
- Investigation of conserved blood or donated plasma to exclude transmission of B19 antigen, since it is highly probable that transmission of B19 virus is no longer possible by anti-B19 IgG positive blood or plasma.
- Selection of anti-B19 positive plasma donors for production of B19 hyperimmunoglobulin products.

There is a pressing need for the introduction of test reagents because of the broad clinical spectrum of the diseases caused by B19, and of the risk to B19-seronegative pregnant women.

It has emerged that utilisable immunologically active polypeptides cannot be prepared directly. Preparation of short peptides by genetic engineering is, just like that of large polypeptides, possible in a satisfactory yield only when suitable expression vectors are used. Although relatively short peptides can be easily prepared by synthesis, more accurate knowledge of the immunological activity is necessary.

The invention relates to immunologically active peptides which have a part of the amino-acid sequence of the capsid proteins VP 1 or VP 2 of parvovirus B19. These peptides are characterised in that they are free of

impurities which interfere with the detection of antibodies directed against parvovirus B19. This property is of great importance since it is not possible to utilise those peptide preparations which contain, by reason of the preparation, components which react with the antibodies to be detected. One example of an unwanted impurity of this type is protein A, which is able to react specifically with the Fc portion of IgG antibodies. A particular advantage of the immunologically active peptides according to the invention is that they can be prepared in good yield by the preparation process according to the invention. This is because, if the antigens required for a diagnostic test are not synthesised in an adequate amount in the preparation process, it is not possible to obtain the required yield after the subsequent purification processes.

It has furthermore been possible within the scope of the present invention to determine short peptide segments from VP 1, more accurately from the region of VP 1 which does not coincide with VP 2, whose epitopes are suitable for reliable detection of antibodies against parvovirus B19 in the investigation fluids, especially sera. This region is called VP 1-VP 2 hereinafter. Fig. 3 shows by way of example the arrangement of some peptides (PAPEP 1-PAPEP 8) in the region (VP 1-VP 2). Although these peptides are preferred, it is equally possible to employ other peptides with 8-50 amino acids, preferably 10 to 32 amino acids, from the VP 1-VP 2 region. This region approximately corresponds to the polypeptide PAN1 which is depicted in Fig. 2-1.

In a preferred embodiment of the present invention, this small, immunodominant and B19-specific region is employed in the serological test. It is particularly preferable in this connection to employ a mixture of synthetic peptides, these peptides having the amino-acid sequences PAPEP 1 - PAPEP 8 shown in Example 3.

In another preferred embodiment of the present invention, the amino-acid sequences which are depicted in

Figure 2 of the immunologically active peptides PAN-1, PAN-2, PAN-3, PAN-4, PCE, PANSE and PAPST prepared by genetic engineering are employed. It is as a rule sufficient in this case to use one peptide in the test. It is possible, however, in special cases also to employ two or more of these peptides.

The peptides according to the invention can be prepared either by synthesis or by genetic engineering. The short peptides, which are explained in detail in Example 3, are preferably prepared by synthesis. The longer peptides are, however, preferably prepared by genetic engineering.

Firstly, the coding regions of the viral DNA were amplified from the serum of an infected patient by means of two polymerase chain reactions (PCR) and cloned in plasmids for further growth in Escherichia (E.) coli. After further subcloning steps, various regions therefrom were then expressed by genetic engineering in E. coli, and the antigens resulting therefrom were investigated for their use for detecting antibodies against the virus. Direct preparation of the peptides according to the invention in expression vectors is impossible because of various difficulties. For this reason, according to the invention, the viral protein segment is fused to a protein amenable to stable expression. This fusion protein can be employed directly after purification as antigen for IgG detection. However, the parvovirus-specific portion is preferably cleaved off by suitable methods, further purified and then employed for serological tests.

The present invention furthermore relates to test kits for the determination of antibodies which are directed against parvovirus B19. The immunologically active peptides according to the invention can in principle be used in all diagnostic test kits for detecting antibodies against parvovirus B19. In a preferred embodiment of the test kits according to the invention, the solid phase of suitable microtitre plates or polystyrene

beads is coated with the immunologically active peptides according to the invention. After incubation with the investigation fluid (serum sample) in a suitable dilution, and after customary washing steps, enzyme- or radioactively labelled anti-human IgG is added. The extent of substrate conversion or of the bound radioactivity then shows whether antibodies directed against parvovirus B19 are present in the serum sample.

The test kits according to the invention are normally supplied to laboratories of physicians, hospitals, investigation facilities etc. They usually contain all the reagents required for carrying out the test. Customary test reagents such as buffer solutions etc. are, however, sometimes not included. As a rule, the test kits contain microtitre plates or polystyrene beads which are coated either with one or more peptides according to the invention or with anti-antibodies. The test kits may furthermore contain, depending on the test principle, one or more peptides according to the invention. Finally, the test kits also embrace an indicator component which makes it possible to quantify the test result.

In other preferred test kits, the antigens are bound to the solid phase of microtitre plates or polystyrene beads. After incubation of the test serum, and suitable washing and saturation steps, a specific enzymatically or radioactively labelled antibody against the B19 antigens is added and its substrate conversion or the bound radioactivity is measured. Since this takes the form of an inhibition test, a small substrate conversion or low radioactivity indicates the presence of specific antibodies.

It is likewise possible to employ peptides according to the invention coupled to solid phases for detecting IgM antibodies against B19. In this detection method, firstly the IgG antibodies are eliminated by adding beads coated with protein A to the investigation fluids. Bound antibodies are then detected using an anti-human IgM antibody which is enzymatically or

radioactively labelled.

The principle of the so-called μ-capture assay is used in another preferred test kit. First the IgM from the investigation fluid (serum) is bound by means of anti-human IgM antibodies bound to the solid phase. The immunologically active peptides according to the invention are then added. The extent of the binding of the antigens and thus the amount of anti-B19 IgM present can be effected by either the antigens being radioactively labelled or labelled with other substances (digoxigenin, avidin) and thus being detectable, or by employing a second labelled antibody against the B19 antigens and measuring its binding.

Very particularly preferred within the scope of the present invention are ELISA (enzyme linked immunosorbent assay) test kits.

Also provided according to the invention are DNA sequences which can be used for direct detection of the virus in investigation samples (sera, biopsies, etc.). Two DNA primers which attach themselves specifically to DNA regions in VP 1 are preferably used. It is then possible by means of a commercially available polymerase chain reaction kit to achieve amplification of the region lying between them. Amplified DNA which has then been immobilised in a suitable way is detected by a suitable DNA sequence. This DNA employed for the hybridisation is prepared with the aid of a plasmid which contains the DNA region lying between the two primers.

It is self-evident that the primer sequences must not be present in the DNA employed for the hybridisation. The sequence of the primers used, and the arrangement with respect to one another, is depicted in Fig. 1.

Finally, vaccines against parvovirus B19 are also made available within the scope of the present invention. This entails the immunologically active peptides according to the invention being administered, optionally several times, together with suitable adjuvants to the people to be protected. The production of antibodies

elicited by this can effect protection from infection with parvovirus B19.

EXAMPLE 1:

Obtaining parvovirus B19 VP 1- and VP 2-encoding sequences from patient's serum

Viral DNA was isolated from 1ml of serum from a patient with acute infection (erythema infectiosum) by proteinase K digestion in 1% SDS, phenol extraction and subsequent alcohol precipitation (this and all the following steps for obtaining, processing and expressing DNA, as well as the preparation of recombinant proteins and fundamental steps for the purification thereof, are described in detail in: Maniatis, T., Fritsch, E.F., Sambrook, J. (1982) Molecular cloning. Cold Spring Harbor, N.Y.). This DNA was taken up in 50µl of TE buffer and then 1µl samples were employed for the amplification by means of the polymerase chain reaction and synthetic oligodeoxynucleotides. Two pairs of primers were used for the amplification of the coding regions of the surface proteins; one of these for obtaining the VP 1 portion, and the second pair for the complete VP p oligodeoxynucleotides used as primers have at each of their 5' ends sequences which are not homologous with the parvovirus sequence, code for restriction enzyme cleavage sites and are therefore suitable for cloning the DNA fragments resulting from the PCR into suitable vectors. The primers identified by O-1 to O-5 in Fig. 1 were used.

In each case five mixtures each containing 1µl of isolated parvovirus DNA were amplified with the two pairs of primers in a volume of 100µl. The conditions for this were: 1.5 min denaturation at 94°C, 2 min attachment of the primers at 45°C, 4 min synthesis at 72°C; total of 50 cycles; buffer, substrates and Taq polymerase were employed for this as stated by the manufacturer (Cetus/Perkin-Elmer, Überlingen, FRG).

The amplified DNA fragments from the two different mixtures (for VP 1 and VP 2) were in each case

combined, precipitated by alcohol precipitation, washed with 70% alcohol, dried, dissolved in a volume of 200µl of TE buffer and digested with the restriction enzymes EcoRI and HindIII. Fractionation of the fragments by electrophoresis in a 1.2% agarose gel was then followed by isolation of the corresponding DNA bands (709bp for VP 1, 1704bp for VP 2) and insertion into the EcoRI and HindIII sites of the vector PUC12 (Pharmacia, Sweden). After transformation of the plasmids into E.coli JM109 (Pharmacia, Sweden), bacterial clones with parvovirus DNA inserts were characterised by restriction digestion. The corresponding zones were given the names pUC12PAN for the region encoding the VP 1 portion and pUC12VP2 for the VP 2-encoding region.

EXAMPLE 2

Preparation by genetic engineering of VP 1 portion and VP 2 from E.coli cells

a) VP1 portion:

1) PAN-1

The VP1-encoding region was isolated from the plasmid pUC12PAN with BclI and HindIII (see Fig. 1, the HindIII site originates from the pUC vector) and inserted behind the 3′ end of a truncated β-galactosidase gene of the vector (for example pBD2) into the BamHI and HindIII restriction cleavage sites. E.coli cells with plasmids resulting therefrom express after induction with IPTG a β-gal::VP1 fusion protein (about 67kDA) in large quantity, which reacts very strongly with anti-parvovirus B19-positive sera in an immunoblot (Western blot). Purification of this protein can be achieved very easily with conventional methods utilising the insolubility of the protein. After lysis of the cells, the pellet fraction is washed with detergents such as Triton™-X100 and octyl gluco-pyranoside, and the fusion protein is subsequently dissolved with 8M urea/1% mercaptoethanol and

separated from cellular impurities by DEAE chromatography with an NaCl gradient.

The VP1 portion can be cleaved off the fusion protein by BrCN cleavage since the VP1 protein sequence starts with a methionine, and this amino acid no longer appears in the fragment itself; by contrast, methionine occurs relatively often in the bacterial fusion portion so that this portion is broken up into very small fragments. After cleavage in 35% formic acid and 0.1mg/ml BrCN at room temperature for 4h, the sample was lyophilised, dissolved in 8M urea, 2mM DTT (dithiothreitol) and purified by DEAE chromatography in an NaCl gradient. The VP1 fragment resulting therefrom was called PAN-1 and can be used directly for serological determinations. The amino-acid sequence is indicated in Fig. 2-1.

Further constructs generated were plasmids which code for fusion proteins consisting of the glutathione S-transferase from Schistosoma japonicum (Smith, D.B. and Johnson, K.S.: Single step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase. Gene, 67 (1988) 31-40) and the VP1 portion. However, it is also possible to use another fusion partner as long as it does not interfere with the diagnostic test.

2) PCE:

The B19 DNA fragment was isolated from pUC12PAN after BclI/PvuII digestion (618bp) and integrated into the BamHI and SmaI sites in pGEX1 (pGEX1PAN). The resulting 52kDA fusion protein was purified from the supernatant by means of glutathione-coupled agarose and employed as antigen for the serological tests in Example 4 (name:PCE). The amino-acid sequence of this antigen is shown in Fig. 2-2.

3) PAN-2:

A 458bp fragment was isolated from pUC12PAN with BclI/HincII and, after intermediate clonings in other vectors, inserted into pGEX2 (pGEX2PAN). Insertion of the fragment in the same reading frame can also be achieved by using oligodeoxynucleotides. At the fusion site of glutathione S-transferase and the VP1 segment is the amino-acid sequence which is recognised by thrombin, so that the B19 portion can be cleaved off the fusion partner by this enzyme. It is also possible to use any other fusion partner as long as it has this protease recognition sequence. The amino-acid sequence of the antigen, as well as fused-on foreign amino acids (in bold print) is indicated in Fig. 2-3.

4) PAN-3:

A 458bp fragment was isolated from pUC12PAN with BclI/HincII and, after intermediate clonings in other vectors, inserted into pGEX3 (pGEX3PAN). Insertion of the fragment in the same reading frame can also be achieved by using synthetic oligodeoxynucleotides. At the fusion site of glutathione S-transferase and the VP1 segment is the amino-acid sequence which is recognised by the protease factor Xa, so that the B19 portion can be cleaved off the fusion partner by this enzyme. It is also possible to use any other fusion partner as long as it has this protease recognition sequence. The amino-acid sequence of the antigen, as well as fused-on foreign amino acids (in bold print) is indicated in Fig. 2-4.

5) PAN-4:

The complete B19 DNA insert was obtained from pUC12PAN by BclI and PstI digestion and, after various intermediate cloning steps, inserted into the vector pGEX2. This resulted in the plasmid pGEX2PAN. Insertion of the fragment in the same reading frame can also be achieved by using synthetic oligodeoxynucleotides. At the fusion site of glutathione S-transferase and the VP1

segment is the amino-acid sequence for the protease thrombin, so that the B19 portion can be cleaved off the fusion partner by this enzyme. It is also possible to use another suitable fusion partner if it has this protease recognition sequence. The amino-acid sequence of the antigen, as well as fused-on foreign amino acids (in bold print) is indicated in Fig. 2-5.

Purification of the antigens can be achieved by simple affinity chromatography with a glutathione-coupled gel matrix.

For further purification of the fusion proteins based on pGEX2 and 3, the B19 portion was cleaved off by digestion with thrombin or factor X as stated by the manufacturer (Boehringer Mannheim). The fragments were then purified again by affinity chromatography. The glutathione S-transferase can in this case be selectively fished out, the parvovirus protein portion is to be found in the flow-through and can be employed after a final DEAE chromatographic fractionation in serological tests.

However, as an alternative to this, the protease can also be added directly to the glutathione-coupled gel suspension with the fusion protein bound on. After an incubation time of about 1h, the VP1 fragment which has been cleaved off can be washed out of the gel, while the glutathione S-transferase portion remains bound to the gel matrix.

b) VP-2 portion:

1) VP-2

Owing to the choice of the PCR primers and of the vector, the coding region for VP2 is already in the correct reading frame in the plasmid pUC12VP2 and can be purified after induction with IPTG from the insoluble fraction of the bacterial lysate, in a similar way to that described for pBD2PAN. The amino-acid sequence of the recombinant antigen is shown in Fig. 2-6.

2) PANSE:

It emerged, surprisingly, that a truncation of the VP2-encoding sequence is associated with a considerable increase in the protein yield, that this truncated antigen can be stably expressed, is not degraded even during purification, and still has the same reactivity with anti-B19 positive sera too. This expression plasmid (pUC19PANSE) was obtained by truncating the 5′ region of VP2 by 355bp as far as an NsiI site. This fragment was inserted into pCU19 (Pharmacia, Sweden) which has the same reading frame in the lacZ peptide as the B19 sequence. Since, because of the PCR primers, a HindIII site is located at the 3′ end, it was necessary also to produce an EcoRI site by intermediate cloning in order to be able to insert the required fragment into the PstI and EcoRI sites of pUC19.

The antigen with a size of about 38kDa (PANSE) can be separated from impurities very simply from the pellet fraction of the bacterial lysate after dissolving in 4M urea by DEAE chromatography. The amino-acid sequence of the antigen is indicated in Fig. 2-7.

3) PAPST:

A fragment 716bp in size which encodes the N-terminal region of VP-2 was isolated from the plasmid pUC12VP2 by PstI digestion. After insertion of the fragment into the vector pUC9 (Pharmacia, Sweden) in the same orientation of the reading frames as the lacZ of the vector (characterised by restriction enzyme digestion), the B19 antigen with a size of about 33kDa is produced in very large quantity (about 10% of the total E.coli protein). Purification can take place in a similar way as for pBDAN from the insoluble constituents by dissolving in 8M urea and subsequent DEAE chromatography. The amino-acid sequence is depicted in Fig. 2-8.

c) Complete VP1/VP2:

The plasmid pUC12PAN was opened with PstI and HindIII, and the VP2 encoding region from pUC12VP2 was inserted after HindIII and partial PstI digestion as 1.7kb fragment (pUC12VP1/2).

Expression of VP1/2-containing antigens in E.coli:

1) PAV-1-B:

pUC12VP1/2 was cut with EcoRI and BamHI, and a DNA band 1466 bp in size was isolated and subsequently inserted into the EcoRI/BamHI sites of the vector pUC18stop. pUC18 stop resembles the abovementioned pUC vectors; however, it differs from the latter by containing between the PstI and HindIII site a synthetic oligodeoxynucleotide which codes for translation stop signals and for the stop of transcription. The polylinker region of the vector thus has the following sequence:

ATG ACC ATG ATT ACG **ATT** TCG AGC TCG GTA CCC GGG **GAT** CCT CTA GAG TCG ACC **TGC** AGT AAT TAA **TTA** GAT CTC GAG CCC GCC TAA TGA GCG GGC TTT TTT **AAG** CTT

(The restriction cleavage sites EcoRI - GAATTC, BamHI - GGATCC, PstI - CTGCAG, BglII - AGATCT and HindIII - AAGCTT are indicated by bold print)

The vector (pUC-V1-B) obtained in this way encodes the VP-1 structural protein from the start up to amino acid 486 followed by some amino acids of the pUC polylinker and is terminated by the stop codon of the inserted oligodeoxynucleotide. The expressed antigen (PAV-1-B) is produced in very good yield after IPTG induction in the E.coli cells and has a size of 60 kDa. Its amino-acid sequence is depicted in Fig. 2-9, amino acids emphasised by bold print are not B19-specific and are encoded by pUC sequences. The reactivity with anti-B19-positive sera is very good and efficient purification can be achieved by removing soluble E.coli proteins, dissolving in 8M urea and conventional ion

exchange chromatography (as described).

2) PAV-1-N:

The vector pUCVP-1-B described above was digested with EcoRI and NsiI. The band 1137bp in size produced in this way was inserted into the vector pUC18stop into the EcoRI and PstI sites (see above). The resulting vector pUCVP-1-N encodes the structural protein from the start up to amino acid 377; the antigen (PAV-1-N) is produced after IPTG induction in the E.coli cells somewhat less well than the antigen PAV-1-B described above. It is 45 kDa in size, and the reactivity with anti-B19 sera is good. The amino-acid sequence is indicated in Fig. 2-10, amino acids with bold print are encoded by the pUC vector and are not B19 specific. Purification of the antigen can be achieved as for PAV-1-B.

3) Expression of the antigens described under c)1) and c)2) as GST fusion proteins

The two vectors pUCVP-1-B and pUCVP-1-N were digested with EcoRI/BglII and the resulting bands about 1480bp and 1150bp, respectively, in size were isolated, with the translation stop signals introduced together with the pUC18stop being transferred too. (The BglII site is indicated in the pUC18stop polylinker sequence indicated above, and the BclI site (TGATCA) immediately before the start of translation was introduced with the primers used for the DNA amplification - see Fig. 1, 0-1). The two fragments which encode the same 5' sequences but regions of different length of VP-1 were then inserted into the vector pGEX-1 described above. Since pGEX-1 has only the SmaI and EcoRI restriction cleavage sites available for insertion of the 3' end of a fragment, it was initially necessary also to produce a site compatible for SmaI (blunt end). This was effected by inserting the two DNA fragments into the EcoRI and BamHI (compatible with BglII) restriction sites of the vector pIC20H (The pIC plasmid and phage vectors with

versatile cloning sites for recombinant selection by insertional inactivation, J.L. Marsh, M. Erfle and E.J. Wykes, Gene, 32 (1984) 481-485). The two fragments were then isolated in turn from the two resulting vectors with BclI and HincII (blunt end cleavage) and inserted into pGEX-1 in BamHI and SmaI. Since HincII also cuts in the B19 sequence, the two fragments were isolated by a partial HincII digestion.

The two pGEX vectors now express fusion proteins consisting of the glutathione S-transferase followed by the two VP-1 segments of different length. The fragment originating from pUCVP-1-B and now located in pGEXVP-1-B yields a fusion protein of about 87kDa; the smaller fragment encoding only up to amino acid 377 a fusion protein 72kDa in size. The amino-acid sequences are indicated in Fig. 2-9 and 2-10. The only difference is that the five N-terminal amino acids are omitted and instead replaced by glutathione S-transferase.

4) Further expression of VP1/VP2:

A 2.4kb fragment was isolated after EcoRI and HindIII digestion from the plasmid pUC12VP1/2 with the complete VP1 and VP2 encoding region and inserted into the eukaryotic expression vector pMDIII (Motz, M., Deby G., Jilg, W., Wolf, H.: Expression of the Epstein-Barr virus major membrane proteins in Chinese hamster ovary cells. Gene, 44 (1986) 353-359. (Obtainable from ATCC)) after EcoRI and HindIII digestion. This plasmid was subsequently linearised again with a SalI, and a 2.4kb SalI fragment with a dihydrofolate reductase gene (DHFR) and regulatory sequences was also inserted. The plasmid pMDIIIVP1/2 obtained in this way was transfected into DHFR-negative CHO cells. Colonies resulting after selection on alpha-minus medium (GIBCO) were isolated and amplified after washing out with increasing concentrations of methotrexate (MTX). Particles with VP1/VP2 can be purified from the culture supernatant from these cell cultures.

Furthermore, the 2.4kb fragment from pUC12VP1/2 was inserted after EcoRI/HindIII digestion into a vector which has, besides the HindIII site, also a BamHI site. The parvovirus portion from this intermediate construct was then isolated as BclI and BamHI fragment and inserted into the BamHI site of a baculovirus expression vector (various constructs can be used). (The BclI site is located immediately in front of the translational start of VP1, it is encoded by the PCR primers, see Fig. 1; the BamHI digestion must be carried out partially since there is also a site of this type still present in the parvovirus sequence.). After co-transfection of the resulting plasmid with wild-type baculovirus DNA into an insect cell culture line, cells which have no so-called inclusion bodies were isolated. The VP1 which is produced intracellularly can be purified in large quantity from those cells in which the baculovirus polyhedrin gene is replaced by the VP1/2 gene.

5) Expression of VP-2:

Furthermore, expression of the smaller B19-VP-2 was obtained using recombinant baculoviruses. For this, the VP-2-encoding plasmid pUC12VP (see Example 1) was digested with EcoRI and HindIII, and the resulting 1.7kb fragment was inserted into an abovementioned vector which has, besides the HindIII site, also a BamHI site. The parvovirus portion was then isolated as BclI and BamHI fragment from this intermediate construct and inserted into the BamHI site of a baculovirus expression vector (various constructs can be used). (The BclI site is located immediately in front of the translational start of BP2, it is encoded by the PCR primers, see Fig. 1, 0-3; the BamHI digestion must be carried out partially since there is also a site of this type still present in the parvovirus sequence). After co-transfection of the resulting plasmid with wild-type baculovirus DNA into an insect cell culture line, cells which have no so-called inclusion bodies were isolated. VP2 can

be purified in large quantity as particles from those cells in which the baculovirus polyhedrin gene is replaced by the VP2 gene. These particles are particularly suitable for use in the μ-capture test.

EXAMPLE 3

Synthetic peptides with immunodominant epitopes

The reaction patterns of the bacterial expression products (especially pGEX::VP1 fusion constructs) with parvovirus-positive sera in a Western blot lead to the surprising conclusion that a short fragment from the VP1 portion suffices to identify all IgG-positive parvo sera.

The fragment can be covered with the following peptides which can be produced by synthesis:

PAPEP-1:
Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr

PAPEP-2:
Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser

PAPEP-3:
Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala

PAPEP-4:
Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val

PAPEP-5:
Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu

PAPEP-6:
Pro Tyr Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe

PAPEP-7:
Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn Ser Ala Glu Ala Ser

PAPEP-8:
Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys His Ile Lys

These antigens can be prepared in large quantities by synthesis without problems, purified and then employed in the ELISA in concentrations between 100-200 ng per mixture.

Even when good results can be achieved with just one of the peptides, conjoint use of two or three peptides is preferred.

It is possible to use for detecting IgG or IgM antibodies in some cases different peptides or combinations thereof.

EXAMPLE 4

a) Determination of serum antibodies against parvovirus B19

The antigens purified and described in Example 2 were used to test a relatively large quantity of sera for their reactivity with these antigens. For this, the various recombinant proteins were added in a concentration of 0.5 - 1 µg/ml in carbonate buffer, pH 9.5, to the wells of commercially available ELISA plates for 16h for binding on. After unbound material has been washed out it is possible then to store these plates in the dry state at 4°C.

Incubation with the sera for 2h took place in a dilution of 1:100, and the subsequent washing procedures

and the detection of bound antibodies with a peroxidase-coupled anti-human IgG antibody took place by conventional test procedures.

Various serum panels were tested for anti-B19 IgG:

1. Sera from a patient with acute B19 infection were investigated consecutively from the appearance of erythema infectiosum up to 19 weeks after the illness.

Result:

All the sera were recognised as anti-B19 IgG positive even from the start of the clinical manifestation and remained positive over the observation period (19 weeks) both with the fusion protein from pGEX1PAN (PCE, see Example 2) and with a VP-1 region cleaved off by BrCN (PAN-1, see Example 2) and with a VP1 portion cleaved off by thrombin (PAN-4) too as antigens.

2. Serum pairs from pregnant women (n=21) from whom a serum sample was taken on hospitalisation and four weeks later were tested for anti-B19 IgG. The same sera were used for each antigen.

Result:

PCE:

Of the 21 pregnant women, 15 were anti-B19 negative and 6 were anti-B19 IgG positive at the time of hospitalisation. The serological result on the second serum sample four weeks later produced an identical result.

PAN-2:

Of the 21 pregnant women, 14 were anti-B19 IgG negative and 7 were anti-B19 positive at the time of hospitalisation. On retesting serum samples taken from these women four weeks later, IgG was no longer detectable in one woman who was previously anti-B19 IgG positive.

PAN-4:

Of the 21 pregnant women, 15 were anti-B19 negative and 6 were anti-B19 IgG positive at the time of hospitalisation. The serological result on the second serum sample four weeks later produced an identical result.

b) Testing of a definitely B19 IgG/M-positive/negative serum collection (n=13)

The sera used were obtained from clinically defined cases and had previously been checked in an IgG/M test which uses purified virus as antigen. Sera 1-6: anti-B19 negative, 7-9: IgM/IgG-positive, 10-13; IgM-negative, IgG-positive.

PAN-4 were tested by the procedure described above. The IgM antibodies were determined by the same test principle as for the IgG determination but PANSE and PAV-1-B were bound as antigens to the plates in a 1:1 mixture with a 10-fold higher concentration, furthermore the serum IgG antibodies were eliminated by pre-adsorption with protein A-coupled beads.

The following values for the absorption were obtained:

IgG determination with PAN-4 (about 20ng per test well), IgM with a 1:1 mixture of PANSE and PAV-1-B (about 150ng per test well total protein)

Serum	IgG	IgM
1	0.09	0.07
2	0.05	0.06
3	0.10	0.08
4	0.07	0.06
5	0.07	0.08
6	0.04	0.07
7	1.82	1.53
8	0.90	0.46
9	0.72	0.56

10	1.10	0.08
11	0.62	0.14
12	0.98	0.11
13	0.87	0.09

The results show a clear discrimination of the positive/negative sera both for the IgG test and for the IgM test.

The IgM-positive sera used were obtained from clinically defined cases and had previously been checked in an IgM test which uses purified virus as antigen. A test mixture with recombinant antigens from the VP1 and VP2 regions also recognised all IgM-positive sera. It emerged that the "PAPST, VP2 but especially PANSE" VP2 portions reacted better in this case than in the IgG test. Both regions will therefore be represented in a commercial test kit for IgM.

A further improvement in the sensitivity can be achieved by selectively binding the serum IgM antibodies to the solid phase by means of monoclonal antibodies, adding recombinant antigen (baculovirus-expressed particulate VP-2) and determining the binding (μ-capture assay).

These experiments demonstrate the high reliability of the test carried out using the immunologically active polypeptides according to the invention.

The VP2 region contained in the antigens called "PANSE, PAPST and VP-2" results in no additional increase in the sensitivity for the determination of antibodies from patients with long-passed infection. On the other hand, a good reaction with these antigens is to be found in the case of sera within infection only in the recent past. This antigen is therefore suitable for providing information about the timing of the infection.

In a test kit it is possible to admix one or a mixture of these antigens either with the VP1 portions produced by genetic engineering or with the synthetic peptide, or else to use these in separate mixtures where the discrimination of the reactivity with these two

regions provides additional information about the timing of the infection.

A further improvement in the sensitivity can be achieved by selective binding of the serum IgM antibodies to the solid phase by means of monoclonal antibodies, adding recombinant antigen and determining the binding (μ-capture assay).

EXAMPLE 5

Use of B19-specific DNA primers for direct detection of pathogen

Any B19 DNA present were obtained from the investigation samples (serum, biopsies) by proteinase K digestion in the presence of 1% SDS (2h, 37°C), phenol extraction and precipitation in 70% ethanol. This, and the DNA amplification which then followed too, was carried out in analogy to the procedure described in Example 1. Primers O-5 and O-2 (see Fig. 1 for the sequence and position on the B19 genome) were used; in the case of B19-positive samples, the amplified fragment has a size of 319bp. Demonstration of the B19-specificity of the DNA fragment was carried out after fractionation of the PCR mixtures by a 1.5% agarose gel, transfer of the DNA to a nitrocellulose membrane (Southern blot) and hybridisation with a piece of DNA which was located between them and which had been labelled either radioactively with P-32 or with digoxigenin by conventional methods (primer extension). The DNA fragment used for the hybridisation was obtained in the following way: a DNA fragment 260 bp long was isolated from the plasmid pUC12PAN after digestion with HincII and PstI and inserted into the HincII and PstI sites in pUC12. It is now possible for the B19 fragment without the sequences used for the amplification to be obtained from the resulting plasmid (pUC12PCRDIA) by EcoRI/PstI digestion and be employed after labelling as hybridisation probe.

In summary, this invention relates to an immunologically active peptide or polypeptide which has part of the amino-acid sequence of the capsid proteins VP1 or VP2 of parvovirus B19, characterised in that it is free of impurities which may interfere with the detection of parvovirus B19 specific antibodies, and the polypeptide is a partial sequence of 8 to 50 amino-acid residues, in particular 10 to 32 amino-acid residues, of the peptide PAN-1, as depicted in Fig. 2-1, or has one or more amino-acid sequences selected from a group consisting of:

Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys His Ile Lys;

Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr;

Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser;

Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala;

Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val;

Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu;

Pro Tyr Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Tly Phe;

Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn Ser Ala Glu Ala Ser;

His Met Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe
Ala Lys Ala Val Tyr Gln Gln Phe Val Glu Phe Tyr Glu Lys Val Thr
Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile
Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val
Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His
His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser
Ser Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser
Ser Glu Asp Leu His Lys Pro Gly Gln Val Ser Val Gln Leu Pro Gly
Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln
Ser Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln
Leu Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp
Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln
Val Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Gly Glu Phe Ile Val
Thr Asp;

Gly Ser Arg Arg Pro Asp His Met Ser Lys Lys Ser Gly Lys Trp Trp
Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val Glu
Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu
Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser
Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser
Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp
His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly
Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val
Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu
Gln Ala Gly Pro Pro Gln Ser Ala Val Gly Asp Pro Arg Glu Phe Ile
Val Thr Asp;

Gly Ile Leu Ser Arg Arg Pro Asp His Met Ser Lys Lys Ser Gly Lys
Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe
Val Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln
Ile Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn
Pro Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys
Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu
Ser Asp His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro
Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly
Gln Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn
Glu Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Gly Asp Pro Leu Glu
Asp Pro Arg Val Pro Ser Ser Asn Ser;

Gly Ser Arg Arg Pro Asp His Met Ser Lys Lys Ser Gly Lys Trp Trp
Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val Glu
Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu
Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser
Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser
Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp
His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly
Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val
Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu
Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile His
Asp Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr Thr
His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu
Thr Gly Phe Gln Ala Gln Val Val Lys Asp Tyr Phe Thr Leu Lys Gly
Ala Ala Ala Pro Val Ala His Phe Gln Gly Ser Leu Pro Glu Val Pro
Ala Tyr Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn Ser
Ala Gly Arg Arg Ile Pro Gly Asn Ser Ser;

Met Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala
Lys Ala Val Tyr Gln Gln Phe Val Glu Phe Tyr Glu Lys Val Thr Gly
Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile Ser
Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala
Arg Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His
Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser Ser
Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser
Glu Asp Leu His Lys Pro Gly Gln Val Ser Val Gln Leu Pro Gly Thr
Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser
Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu
Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu
Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Val
Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Ala Ala Pro Val Ala His
Phe Gln Gly Ser Leu Pro Glu Val Pro Ala Tyr Asn Ala Ser Glu Lys
Tyr Pro Ser;

or partial sequences thereof

or selected from a group consisting of:

Met Thr Ile Thr Asn Ser Asp His Met Ser Lys Lys Ser Gly Lys Trp
Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val
Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile
Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro
Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn
Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser
Asp His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg
Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln
Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu
Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile
His Asp Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr
Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn
Glu Thr Gly Phe Gln Ala Gln Val Val Lys Asp Tyr Phe Thr Leu Lys
Gly Ala Ala Ala Pro Val Ala His Phe Gln Gly Ser Leu Pro Glu Val
Pro Ala Tyr Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn
Ser Ala Glu Ala Ser Thr Gly Ala Gly Gly Gly Gly Ser Asn Ser Val
Lys Ser Met Trp Ser Glu Gly Ala Thr Phe Ser Ala Asn Ser Val Thr
Cys Thr Phe Ser Arg Gln Phe Leu Ile Pro Tyr Asp Pro Glu His His
Tyr Lys Val Phe Ser Pro Ala Ala Ser Ser Cys His Asn Ala Ser Gly
Lys Glu Ala Lys Val Cys Thr Ile Ser Pro Ile Met Gly Tyr Ser Thr
Pro Trp Arg Tyr Leu Asp Phe Asn Ala Leu Asn Leu Phe Phe Ser Pro
Leu Glu Phe Gln His Leu Ile Glu Asn Tyr Gly Ser Ile Ala Pro Asp
Ala Leu Thr Val Thr Ile Ser Glu Ile Ala Val Lys Asp Val Thr Asp
Lys Thr Gly Gly Gly Val Gln Val Thr Asp Ser Thr Thr Gly Arg Leu
Cys Met Leu Val Asp His Glu Tyr Lys Tyr Pro Tyr Val Leu Gly Gln
Gly Gln Asp Thr Leu Ala Pro Glu Leu Pro Ile Trp Val Tyr Phe Pro
Pro Gln Tyr Ala Tyr Leu Thr Val Gly Asp Val Asn Thr Gln Gly Ile
Ser Gly Asp Ser Lys Lys Leu Ala Ser Glu Glu Ser Ala Phe Tyr Val
Leu Glu His Ser Ser Phe Gln Leu Leu Gly Thr Gly Gly Thr Ala Ser
Met Ser Tyr Lys Phe Pro Pro Val Pro Pro Glu Asn Leu Glu Gly Cys
Ser Gln His Phe Tyr Glu Met Tyr Asn Pro Leu Tyr Gly Ser Ser Arg
Val Asp Leu Gln;

Met Thr Ile Thr Asn Ser Asp His Met Ser Lys Lys Ser Gly Lys Trp
Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val
Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile
Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro
Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn
Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser
Asp His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg
Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln
Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu
Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile
His Asp Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr
Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn
Glu Thr Gly Phe Gln Ala Gln Val Val Lys Asp Tyr Phe Thr Leu Lys
Gly Ala Ala Ala Pro Val Ala His Phe Gln Gly Ser Leu Pro Glu Val
Pro Ala Tyr Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn
Ser Ala Glu Ala Ser Thr Gly Ala Gly Gly Gly Gly Ser Asn Ser Val
Lys Ser Met Trp Ser Glu Gly Ala Thr Phe Ser Ala Asn Ser Val Thr
Cys Thr Phe Ser Arg Gln Phe Leu Ile Pro Tyr Asp Pro Glu His His
Tyr Lys Val Phe Ser Pro Ala Ala Ser Ser Cys His Asn Ala Ser Gly
Lys Glu Ala Lys Val Cys Thr Ile Ser Pro Ile Met Gly Tyr Ser Thr
Pro Trp Arg Tyr Leu Asp Phe Asn Ala Leu Asn Leu Phe Phe Ser Pro
Leu Glu Phe Gln His Leu Ile Glu Asn Tyr Gly Ser Ile Ala Pro Asp
Ala Leu Thr Val Thr Ile Ser Glu Ile Ala Val Lys Asp Val Thr Asp
Lys Thr Gly Gly Gly Val Gln Val Thr Asp Ser Thr Thr Gly Arg Leu
Cys Ser Asn

This invention further relates to a test kit for the detection of antibodies against human parvovirus B19, characterised in that it has at least one immunologically active peptide or polypeptide according which is able to react with the antibodies present in the investigation fluids, and in that it has at least one indicator component which makes it possible to detect complexes of immunologically active peptide and antibody.

This invention further relates to a process for the purification of immunologically active peptides or polypeptides characterised in that it comprises the dissolving of unpurified peptide or polypeptide in 8M urea, and fractionation by a DEAE Sephacel™ column with an NaCl gladient.

This invention further relates to the use of at least one DNA sequence selected from 0-1:

GTG AAT TCT GAT CAT ATG AGT AAA AAA AGT GGC AAA TGG

0-2:

C TTC GGT CGT GAC CAC GTC CTC CCC

0-3:

G AGG AAT TCT CTG ATC ATG ACT TCA GTT AAT TCT GCA GAA GCC

0-4:

GAG GGG TGG CAC GGG ACT CGG TCC TTC GAA GAG

0-5:

G CTA CAA GCT GGG CCC CCG CAA AG

for the direct detection of parvovirus B19 by means of DNA amplification, especially by means of polymerase chain reaction.

This invention further relates to the use of immunologically active peptides as vaccines against infections with parvovirus B19.

DESCRIPTION OF THE FIGURES

Fig. 1: Diagrammatic representation of the VP1/2 encoding region of parvovirus B19 with the primer sequences used for the amplification.

The structure of the single-stranded B19 genome with the inverse regions at the ends (double strand) and with coding regions is depicted diagrammatically in the upper part. The coding region for the non-structural proteins (NS) which are synthesised as polypeptide and then processed is in the left region. The right region codes for the surface proteins of the viral capsid (VP1/2), with VP1 being, apart from an additional N-terminal region (shaded bar), identical to VP2 (black bar). Underneath this are indicated the regions of oligodeoxynucleotides O-1 to O-4 which were used as primers for the amplification (PCR) of the B19 sequences located between them (O-1 and O-2 for the VP1 region, and O-3 and O-4 for VP-2).

The DNA sequences of the corresponding B19 regions as well as of the oligodeoxynucleotides are indicated in the lower part of the figure. The oligodeoxynucleotide sequences are identified by bold print, non-homologous regions, that is to say sequences which do not hybridise with B19, are contrasted by a line spacing. These non-hybridising sequences represent restriction enzymes sites for EcoRI (GAATTC) and BclI (TGATCA) in the case of O-1, for EcoRI, BclI and BspHII (TC-ATGA) in the case of O-3, and for HindIII (AAGCT-T) in the case of O-4. The amplified VP2 encoding fragment (O-3 and O-4) was digested with EcoRI and HindIII before insertion in pUC vectors, the VP1 encoding fragment with EcoRI and PstI, the PstI cleavage site being located in the B19 DNA (from position no. 4 in the indicated sequence for O-2, CTGCAG).

Fig. 2: Amino-acid sequences of the antigens described in Example 2 and produced by recombination in E.coli cells.

Owing to cloning steps, in each case some non-B19-authentic foreign amino acids are also contained at the N-termini and at the C-termini (apart from PANSE and VP-2) and are emphasised by bold print.

The amino-acid sequences of the antigens described in Example 2 are described:

Fig. 2-1: PAN-1
Fig. 2-2: PCE
Fig. 2-3: PAN-2
Fig. 2-4: PAN-3
Fig. 2-5: PAN-4
Fig. 2-6: VP2
Fig. 2-7: PANSE
Fig. 2-8: PAPST
Fig. 2-9: PAV-1B
Fig. 2-10: PAV-1N

Fig. 3: Diagrammatic representation of the arrangement of some peptides with respect to one another

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Immunologically active peptide or polypeptide which has a part of the amino-acid sequence of the capsid proteins VP1 or VP2 of parvovirus B19, characterised in that it is free of impurities which may interfere with the detection of parvovirus B19 specific antibodies, and the polypeptide is a partial sequence of 8 to 50 amino-acid residues of the peptide PAN-1, as depicted in Fig. 2-1, or has one or more amino-acid sequences selected from a group consisting of:

Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys His Ile Lys;

Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr;

Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser;

Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala;

Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val;

Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu;

Pro Tyr Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Tly Phe;

Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn Ser Ala Glu Ala Ser;

His Met Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe
Ala Lys Ala Val Tyr Gln Gln Phe Val Glu Phe Tyr Glu Lys Val Thr
Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile
Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val
Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His
His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser
Ser Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser
Ser Glu Asp Leu His Lys Pro Gly Gln Val Ser Val Gln Leu Pro Gly
Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln
Ser Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln
Leu Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp
Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln
Val Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Gly Glu Phe Ile Val
Thr Asp;

Gly Ser Arg Arg Pro Asp His Met Ser Lys Lys Ser Gly Lys Trp Trp
Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val Glu
Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu
Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser
Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser
Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp
His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly
Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val
Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu
Gln Ala Gly Pro Pro Gln Ser Ala Val Gly Asp Pro Arg Glu Phe Ile
Val Thr Asp;

Gly Ile Leu Ser Arg Arg Pro Asp His Met Ser Lys Lys Ser Gly Lys
Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe
Val Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln
Ile Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn
Pro Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys
Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu
Ser Asp His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro
Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly
Gln Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn
Glu Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Gly Asp Pro Leu Glu
Asp Pro Arg Val Pro Ser Ser Asn Ser;

Gly Ser Arg Arg Pro Asp His Met Ser Lys Lys Ser Gly Lys Trp Trp
Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val Glu
Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu
Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser
Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser
Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp
His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly
Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val
Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu
Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile His
Asp Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr Thr
His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu
Thr Gly Phe Gln Ala Gln Val Val Lys Asp Tyr Phe Thr Leu Lys Gly
Ala Ala Ala Pro Val Ala His Phe Gln Gly Ser Leu Pro Glu Val Pro
Ala Tyr Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn Ser
Ala Gly Arg Arg Ile Pro Gly Asn Ser Ser;

and partial sequences thereof.

2. Immunologically active peptide or polypeptide which has a part of the amino-acid sequence of the capsid proteins VP1 or VP2 of parvovirus B19, characterised in that it is free of impurities which may interfere with the detection of parvovirus B19 specific antibodies, and the peptide is PAN-1, as depicted in Fig. 2-1.

3. Immunologically active peptide or polypeptide which has a part of the amino-acid sequence of the capsid proteins VP1 or VP2 of parvovirus B19, characterised in that it is free of impurities which may interfere with the detection of parvovirus B19 specific antibodies, and the polypeptide is a partial sequence of 10 to 32 amino-acid residues of the peptide PAN-1, as depicted in Fig. 2-1, or has one or more amino-acid sequences selected from a group consisting of:

Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys His
Ile Lys;

Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr;

Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser;

Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala;

Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val;

Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu;

Pro Tyr Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Tly Phe;

Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn Ser Ala Glu Ala Ser;

His Met Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Val Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Gly Glu Phe Ile Val Thr Asp;

Gly Ser Arg Arg Pro Asp His Met Ser Lys Lys Ser Gly Lys Trp Trp
Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val Glu
Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu
Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser
Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser
Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp
His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly
Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val
Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu
Gln Ala Gly Pro Pro Gln Ser Ala Val Gly Asp Pro Arg Glu Phe Ile
Val Thr Asp;

Gly Ile Leu Ser Arg Arg Pro Asp His Met Ser Lys Lys Ser Gly Lys
Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe
Val Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln
Ile Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn
Pro Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys
Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu
Ser Asp His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro
Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly
Gln Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn
Glu Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Gly Asp Pro Leu Glu
Asp Pro Arg Val Pro Ser Ser Asn Ser;

Gly Ser Arg Arg Pro Asp His Met Ser Lys Lys Ser Gly Lys Trp Trp
Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val Glu
Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu
Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser
Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser
Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp
His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly
Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val
Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu
Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile His
Asp Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr Thr
His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu
Thr Gly Phe Gln Ala Gln Val Val Lys Asp Tyr Phe Thr Leu Lys Gly
Ala Ala Ala Pro Val Ala His Phe Gln Gly Ser Leu Pro Glu Val Pro

Ala Tyr Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn Ser Ala Gly Arg Arg Ile Pro Gly Asn Ser Ser;

and partial sequences thereof.

4. Immunologically active peptide or polypeptide which has a part of the amino-acid sequence of the capsid proteins VP1 or VP2 of parvovirus B19, characterised in that it is free of impurities which may interfere with the detection of parvovirus B19 specific antibodies, and the polypeptide has one or more amino-acid sequences selected from a group consisting of:

Met Thr Ile Thr Asn Ser Asp His Met Ser Lys Lys Ser Gly Lys Trp
Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val
Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile
Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro
Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn
Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser
Asp His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg
Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln
Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu
Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile
His Asp Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr
Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn
Glu Thr Gly Phe Gln Ala Gln Val Val Lys Asp Tyr Phe Thr Leu Lys
Gly Ala Ala Ala Pro Val Ala His Phe Gln Gly Ser Leu Pro Glu Val
Pro Ala Tyr Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn
Ser Ala Glu Ala Ser Thr Gly Ala Gly Gly Gly Gly Ser Asn Ser Val
Lys Ser Met Trp Ser Glu Gly Ala Thr Phe Ser Ala Asn Ser Val Thr
Cys Thr Phe Ser Arg Gln Phe Leu Ile Pro Tyr Asp Pro Glu His His
Tyr Lys Val Phe Ser Pro Ala Ala Ser Ser Cys His Asn Ala Ser Gly
Lys Glu Ala Lys Val Cys Thr Ile Ser Pro Ile Met Gly Tyr Ser Thr
Pro Trp Arg Tyr Leu Asp Phe Asn Ala Leu Asn Leu Phe Phe Ser Pro
Leu Glu Phe Gln His Leu Ile Glu Asn Tyr Gly Ser Ile Ala Pro Asp
Ala Leu Thr Val Thr Ile Ser Glu Ile Ala Val Lys Asp Val Thr Asp
Lys Thr Gly Gly Gly Val Gln Val Thr Asp Ser Thr Thr Gly Arg Leu
Cys Met Leu Val Asp His Glu Tyr Lys Tyr Pro Tyr Val Leu Gly Gln

Gly Gln Asp Thr Leu Ala Pro Glu Leu Pro Ile Trp Val Tyr Phe Pro
Pro Gln Tyr Ala Tyr Leu Thr Val Gly Asp Val Asn Thr Gln Gly Ile
Ser Gly Asp Ser Lys Lys Leu Ala Ser Glu Glu Ser Ala Phe Tyr Val
Leu Glu His Ser Ser Phe Gln Leu Leu Gly Thr Gly Gly Thr Ala Ser
Met Ser Tyr Lys Phe Pro Pro Val Pro Pro Glu Asn Leu Glu Gly Cys
Ser Gln His Phe Tyr Glu Met Tyr Asn Pro Leu Tyr Gly Ser Ser Arg
Val Asp Leu Gln; and

Met Thr Ile Thr Asn Ser Asp His Met Ser Lys Lys Ser Gly Lys Trp
Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val
Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile
Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro
Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn
Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser
Asp His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg
Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln
Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu
Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile
His Asp Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr
Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn
Glu Thr Gly Phe Gln Ala Gln Val Val Lys Asp Tyr Phe Thr Leu Lys
Gly Ala Ala Ala Pro Val Ala His Phe Gln Gly Ser Leu Pro Glu Val
Pro Ala Tyr Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn
Ser Ala Glu Ala Ser Thr Gly Ala Gly Gly Gly Gly Ser Asn Ser Val
Lys Ser Met Trp Ser Glu Gly Ala Thr Phe Ser Ala Asn Ser Val Thr
Cys Thr Phe Ser Arg Gln Phe Leu Ile Pro Tyr Asp Pro Glu His His
Tyr Lys Val Phe Ser Pro Ala Ala Ser Ser Cys His Asn Ala Ser Gly
Lys Glu Ala Lys Val Cys Thr Ile Ser Pro Ile Met Gly Tyr Ser Thr
Pro Trp Arg Tyr Leu Asp Phe Asn Ala Leu Asn Leu Phe Phe Ser Pro
Leu Glu Phe Gln His Leu Ile Glu Asn Tyr Gly Ser Ile Ala Pro Asp
Ala Leu Thr Val Thr Ile Ser Glu Ile Ala Val Lys Asp Val Thr Asp
Lys Thr Gly Gly Gly Val Gln Val Thr Asp Ser Thr Thr Gly Arg Leu
Cys Ser Asn.

5. Immunologically active peptide or polypeptide according to Claim 1, 2, 3 or 4, characterised in that it is in the form of a fusion protein, where this fusion

protein has at least a part of β-galactosidase or of glutathione S-transferase.

6. Test kit for the detection of antibodies against human parvovirus B19, characterised in that it has at least one immunologically active peptide or polypeptide according to Claim 1, 2, 3 or 4, which is able to react with the antibodies present in the investigation fluids, and in that it has at least one indicator component which makes it possible to detect complexes of immunologically active peptide and antibody.

7. Test kit according to Claim 6, characterised in that the indicator component is an antibody which is directed against the antibody to be detected and has a label.

8. Test kit according to Claim 7, characterised in that the label consists of a radioactive isotope.

9. Test kit according to Claim 7, characterised in that the label consists of an enzyme which is able to catalyse a colour reaction.

10. Test kit according to Claim 7, characterised in that the immunologically active peptide or polypeptide is biotinylated, and the indicator component is avidin or streptavidin with enzyme covalently bonded thereto.

11. Test kit according to Claim 10, characterised in that peroxidase is covalently bonded to said indicator component.

12. Test kit according to any of Claims 7 to 11, characterised in that it is an ELISA kit.

13. Test kit according to Claim 12, characterised in that at least one immunologically active peptide or polypeptide according to Claim 1, 2, 3 or 4 is coupled to microtitre plates, and in that the indicator component consists of anti-human IgG and/or IgM antibodies to which an enzyme catalysing a colour reaction is coupled.

14. Test kit according to Claim 12, characterised in that monoclonal antibodies against human IgM antibodies are coupled to microtitre plates, and in that the indicator component is a biotinylated immunologically active peptide or polypeptide according to Claim 1, 2, 3 or 4 which cooperates with avidin or streptavidin with enzyme covalently bonded thereto.

15. Process for the purification of immunologically active peptides or polypeptides according to Claim 1, 2, 3 or 4 characterised in that it comprises the dissolving of unpurified peptide or polypeptide in 8M urea, and fractionation by a DEAE Sephacel™ column with an NaCl gladient.

16. Process according to Claim 15, additionally comprising an affinity chromatography.

17. Process according to Claim 16, characterised in that the affinity chromatography is carried out with a glutathione-coupled gel matrix.

18. Use of at least one DNA sequence selected from

0-1:

GTG AAT TCT GAT CAT ATG AGT AAA AAA AGT GGC AAA TGG

0-2:

C TTC GGT CGT GAC CAC GTC CTC CCC

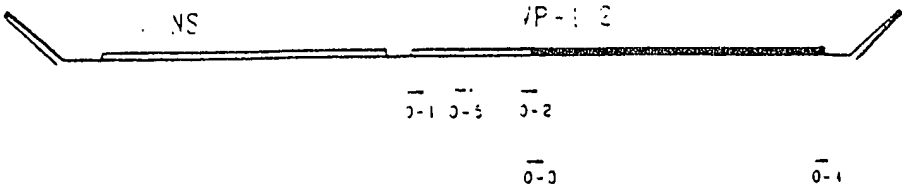
0-3:

G AGG AAT TCT CTG ATC ATG ACT TCA GTT AAT TCT GCA GAA GCC

0-4:
GAG GGG TGG CAC GGG ACT CGG TCC TTC GAA GAG and
0-5:
G CTA CAA GCT GGG CCC CCG CAA AG
for the direct detection of parvovirus B19 by means of DNA amplification.

19. Use of at least one DNA sequence as claimed in claim 18, for the direct detection of parvovirus B19 by means of polymerase chain reaction.

20. Use of immunologically active peptides according to Claim 1, 2, 3 or 4 as vaccine against infections with parvovirus B19.



O-1:

5'GTG AAT TCT GAT CAT
ATG AGT AAA AAA AGT GGC AAA TGG3'
5'AAA GCT TTG TAG ATT ATG AGT AAA AAA AGT GGC AAA TGG TGG3'
3'TTT CGA AAC ATC TAA TAC TCA TTT TTT TCA CCG TTT ACC ACC5'

O-2:

5'ATT CTG CAG AAG CCA GCA CTG GTG CAG GAG GGG GGG GCA3'
3'TAA GAC GTC TTC GGT CGT GAC CAC GTC CTC CCC CCC CGT5'
3'C TTC GGT CGT GAC CAC GTC CTC CCC5'

O-3:

5'G AGG AAT TCT CTG ATC
ATG ACT TCA GTT AAT TCT GCA GAA GCC3'
5'A GAA AAA TAC CGA AGC ATG ACT TCA GTT AAT TCT GCA GAA GCC3'
3'T CTT TTT ATG GCT TCG TAC TGA AGT CAA TTA AGA CGT CTT CGG5'

O-4:

5'TTG TAA ACA CTC CCC ACC GTG CCC TCA GCC AGG ATG CGT A3'
3'AAC ATT TGT GAG GGG TCC CAC GGG AGT CGG TCC TAC GCA T5'
3'GAG GGG TGG CAC GGG AGT CGG TCC T
TC GAA GAG5'

O-5

5'G CTA CAA GCT GGG CCC CCG CAA AG3'
5'GAG CTA CAA GCT GGG CCC CCG CAA AGT GCT GTT GAC AGT GCT3'
3'CAC GAT GTT CGA CCC GGG GGC GTT TCA CGA CAA CTG TCA CGA5'

FIG. 1

Fig. 2

Fig. 2-1

PAN-1:

Met Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Val Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Ala Ala Pro Val Ala His Phe Gln Gly Ser Leu Pro Glu Val Pro Ala Tyr Asn Ala Ser Glu Lys Tyr Pro Ser

Fig. 2-2

PCE:

Gluthauon-S-Transferase -His Met Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Val Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Gly Glu Phe Ile Val Thr Asp

Fig. 2-3

PAN-2

Gly Ser Arg Arg Pro Asp His Met Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Gly Asp Pro Arg Glu Phe Ile Val Thr Asp

Fig. 2-4

PAN-3

Gly Ile Leu Ser Arg Arg Pro Asp His Met Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala
Lys Ala Val Tyr Gln Gln Phe Val Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu
Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala Arg Ile
Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro
His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His
Lys Pro Gly Gln Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro
Pro Gln Ser Ala Val Gly Asp Pro Leu Glu Asp Pro Arg Val Pro Ser Ser Asn Ser

Fig. 2-5

PAN-4

Gly Ser Arg Arg Pro Asp His Met Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala
Val Tyr Gln Gln Phe Val Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp
His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn
Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala
Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro
Gly Gln Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln
Ser Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr
Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Val Val
Lys Asp Tyr Phe Thr Leu Lys Gly Ala Ala Ala Pro Val Ala His Phe Gln Gly Ser Leu Pro Glu Val Pro Ala
Tyr Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn Ser Ala Gly Arg Arg Ile Pro Gly Asn Ser Ser

Fig. 2-6

VP2:

Met Thr Met Ile Thr Asn Ser Leu Ile Met Thr Ser Val Asn Ser Ala Glu Ala Ser Thr Gly Ala Gly Gly Gly
Gly Ser Asn Ser Val Lys Ser Met Trp Ser Glu Gly Ala Thr Phe Ser Ala Asn Ser Val Thr Cys Thr Phe Ser
Arg Gln Phe Leu Ile Pro Tyr Asp Pro Glu His His Tyr Lys Val Phe Ser Pro Ala Ala Ser Ser Cys His Asn
Ala Ser Gly Lys Glu Ala Lys Val Cys Thr Ile Ser Pro Ile Met Gly Tyr Ser Thr Pro Trp Arg Tyr Leu Asp Phe
Asn Ala Leu Asn Leu Phe Phe Ser Pro Leu Glu Phe Gln His Leu Ile Glu Asn Tyr Gly Ser Ile Ala Pro Asp
Ala Leu Thr Val Thr Ile Ser Glu Ile Ala Val Lys Asp Val Thr Asp Lys Thr Gly Gly Gly Val Gln Val Thr
Asp Ser Thr Thr Gly Arg Leu Cys Met Leu Val Asp His Glu Tyr Lys Tyr Pro Tyr Val Leu Gly Gln Gly Gln
Asp Thr Leu Ala Pro Glu Leu Pro Ile Trp Val Tyr Phe Pro Pro Gln Tyr Ala Tyr Leu Thr Val Gly Asp Val
Asn Thr Gln Gly Ile Ser Gly Asp Ser Lys Lys Leu Ala Ser Glu Glu Ser Ala Phe Tyr Val Leu Glu His Ser
Ser Phe Gln Leu Leu Gly Thr Gly Gly Thr Ala Ser Met Ser Tyr Lys Phe Pro Pro Val Pro Pro Glu Asn Leu
Glu Gly Cys Ser Gln His Phe Tyr Glu Met Tyr Asn Pro Leu Tyr Gly Ser Arg Leu Gly Val Pro Asp Thr Leu
Gly Gly Asp Pro Lys Phe Arg Ser Leu Thr His Glu Asp His Ala Ile Gln Pro Gln Asn Phe Met Pro Gly Pro
Leu Val Asn Ser Val Ser Thr Lys Glu Gly Asp Ser Ser Asn Thr Gly Ala Gly Lys Ala Leu Thr Gly Leu Ser
Thr Gly Thr Ser Gln Asn Thr Arg Ile Ser Leu Arg Pro Gly Pro Val Ser Gln Pro Tyr His His Trp Asp Thr
Asp Lys Tyr Val Thr Gly Ile Asn Ala Ile Ser His Gly Gln Thr Thr Tyr Gly Asn Ala Glu Asp Lys Glu Tyr
Gln Gln Gly Val Gly Arg Phe Pro Asn Glu Lys Glu Gln Leu Lys Gln Leu Gln Gly Leu Asn Met His Thr
Tyr Phe Pro Asn Lys Gly Thr Gln Gln Tyr Thr Asp Gln Ile Glu Arg Pro Leu Met Val Gly Ser Val Trp Asn
Arg Arg Ala Leu His Tyr Glu Ser Gln Leu Trp Ser Lys Ile Pro Asn Leu Asp Asp Ser Phe Lys Thr Gln Phe
Ala Ala Leu Gly Gly Trp Gly Leu His Gln Pro Pro Pro Gln Ile Phe Leu Lys Gln Tyr Ala Val Gly Ile Met
Thr Val Thr Met Thr Phe Lys Leu Gly Pro Arg Lys Ala Thr Gly Arg Trp Asn Pro Gln Pro Gly Val Tyr Pro
Pro His Ala Ala Gly His Leu Pro Thr Val Leu Tyr Asp Pro Thr Ala Thr Asp Ala Lys Gln His His Arg His
Gly Tyr Glu Lys Pro Glu Glu Leu Trp Thr Ala Lys Ser Arg Val His Pro Leu

Fig. 2-7

PANSE:

Met Thr Met Ile Thr Pro Ser Leu His Ala Cys Met Leu Val Asp His Glu Tyr Lys Tyr Pro Tyr Val Leu Gly Gln Gly Gln Asp Thr Leu Ala Pro Glu Leu Pro Ile Trp Val Tyr Phe Pro Pro Gln Tyr Ala Tyr Leu Thr Val Gly Asp Val Asn Thr Gln Gly Ile Ser Gly Asp Ser Lys Lys Leu Ala Ser Glu Glu Ser Ala Phe Tyr Val Leu Glu His Ser Ser Phe Gln Leu Leu Gly Thr Gly Gly Thr Ala Ser Met Ser Tyr Lys Phe Pro Pro Val Pro Pro Glu Asn Leu Glu Gly Cys Ser Gln His Phe Tyr Glu Met Tyr Asn Pro Leu Tyr Gly Ser Arg Leu Gly Val Pro Asp Thr Leu Gly Gly Asp Pro Lys Phe Arg Ser Leu Thr His Glu Asp His Ala Ile Gln Pro Gln Asn Phe Met Pro Gly Pro Leu Val Asn Ser Val Ser Thr Lys Glu Gly Asp Ser Ser Asn Thr Gly Ala Gly Lys Ala Leu Thr Gly Leu Ser Thr Gly Thr Ser Gln Asn Thr Arg Ile Ser Leu Arg Pro Gly Pro Val Ser Gln Pro Tyr His His Trp Asp Thr Asp Lys Tyr Val Thr Gly Ile Asn Ala Ile Ser His Gly Gln Thr Thr Tyr Gly Asn Ala Glu Asp Lys Glu Tyr Gln Gln Gly Val Gly Arg Phe Pro Asn Glu Lys Glu Gln Leu Lys Gln Leu Gln Gly Leu Asn Met His Thr Tyr Phe Pro Asn Lys Gly Thr Gln Gln Tyr Thr Asp Gln Ile Glu Arg Pro Leu Met Val Gly Ser Val Trp Asn Arg Arg Ala Leu His Tyr Glu Ser Gln Leu Trp Ser Lys Ile Pro Asn Leu Asp Asp Ser Phe Lys Thr Gln Phe Ala Ala Leu Gly Gly Trp Gly Leu His Gln Pro Pro Pro Gln Ile Phe Leu Lys Gln Tyr Ala Val Gly Ile Met Thr Val Thr Met Thr Phe Lys Leu Gly Pro Arg Lys Ala Thr Gly Arg Trp Asn Pro Gln Pro Gly Val Tyr Pro Pro His Ala Ala Gly His Leu Pro Tyr Val Leu Tyr Asp Pro Thr Ala Thr Asp Ala Lys Gln His His Arg His Gly Tyr Glu Lys Pro Glu Glu Leu Trp Thr Ala Lys Ser Arg Val His Pro Leu

Fig. 2-8

PAPST:

Met Thr Met Ile Thr Pro Ser Leu Ala Ala Glu Ala Ser Thr Gly Ala Gly Gly Gly Gly Ser Asn Ser Val Lys Ser Met Trp Ser Glu Gly Ala Thr Phe Ser Ala Asn Ser Val Thr Cys Thr Phe Ser Arg Gln Phe Leu Ile Pro Tyr Asp Pro Glu His His Tyr Lys Val Phe Ser Pro Ala Ala Ser Ser Cys His Asn Ala Ser Gly Lys Glu Ala Lys Val Cys Thr Ile Ser Pro Ile Met Gly Tyr Ser Thr Pro Trp Arg Tyr Leu Asp Phe Asn Ala Leu Asn Leu Phe Phe Ser Pro Leu Glu Phe Gln His Leu Ile Glu Asn Tyr Gly Ser Ile Ala Pro Asp Ala Leu Thr Val Thr Ile Ser Glu Ile Ala Val Lys Asp Val Thr Asp Lys Thr Gly Gly Gly Val Gln Val Thr Asp Ser Thr Thr Gly Arg Leu Cys Met Leu Val Asp His Glu Tyr Lys Tyr Pro Tyr Val Leu Gly Gln Gly Gln Asp Thr Leu Ala Pro Glu Leu Pro Ile Trp Val Tyr Phe Pro Pro Gln Tyr Ala Tyr Leu Thr Val Gly Asp Val Asn Thr Gln Gly Ile Ser Gly Asp Ser Lys Lys Leu Ala Ser Glu Glu Ser Ala Phe Tyr Val Leu Glu His Ser Ser Phe Gln Leu Leu Gly Thr Gly Gly Thr Ala Ser Met Ser Tyr Lys Phe Pro Pro Val Pro Pro Glu Asn Leu Glu Gly Cys Arg Ser Thr Asp Pro Arg Glu Phe Thr Gly Arg Arg Phe Thr Thr Ser

Fig. 2-9

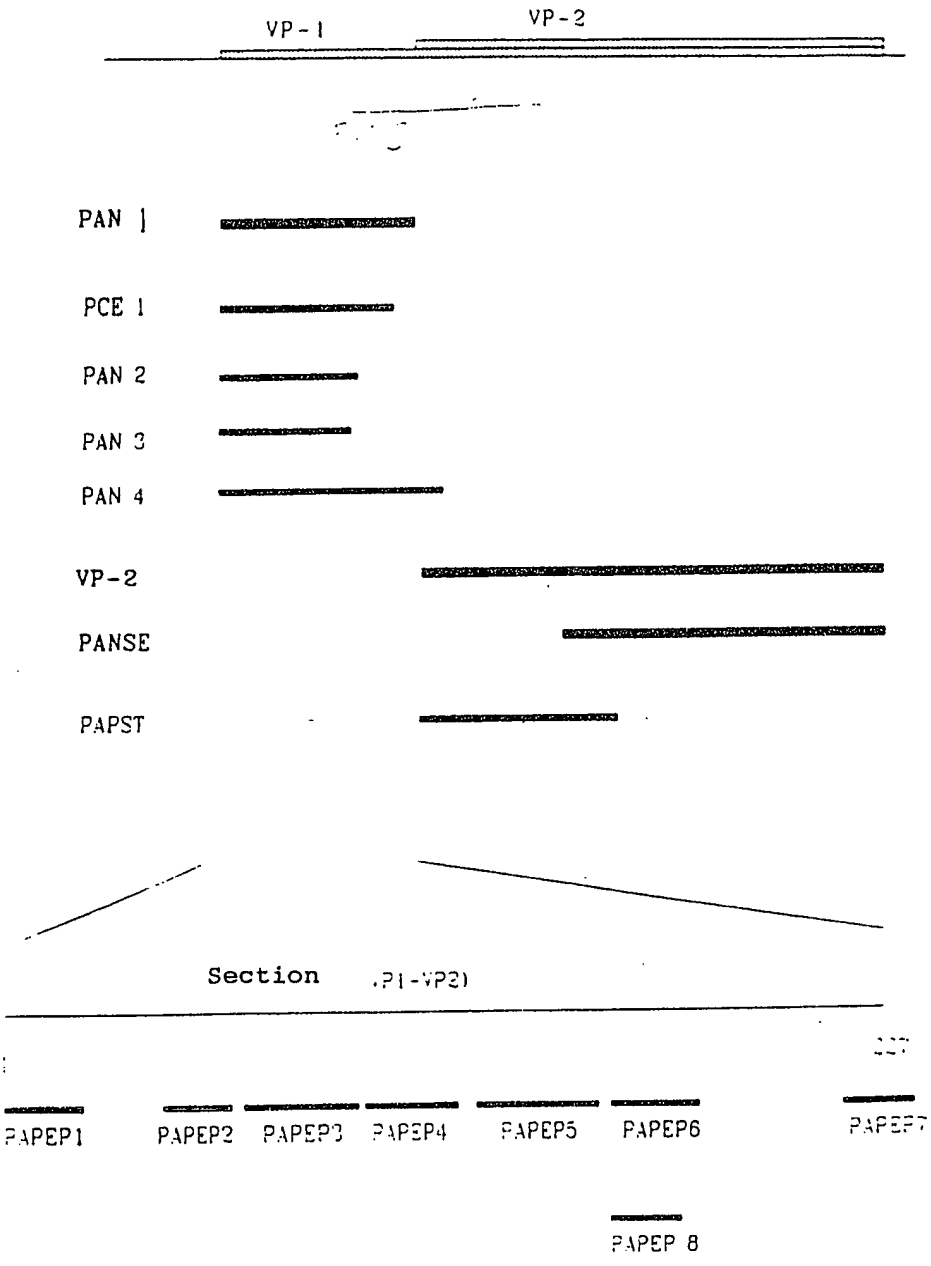
PAV-1-B

Met Thr Ile Thr Asn Ser Asp His Met Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Val Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Ala Ala Pro Val Ala His Phe Gln Gly Ser Leu Pro Glu Val Pro Ala Tyr Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn Ser Ala Glu Ala Ser Thr Gly Ala Gly Gly Gly Gly Gly Ser Asn Ser Val Lys Ser Met Trp Ser Glu Gly Ala Thr Phe Ser Ala Asn Ser Val Thr Cys Thr Phe Ser Arg Gln Phe Leu Ile Pro Tyr Asp Pro Glu His His Tyr Lys Val Phe Ser Pro Ala Ala Ser Ser Cys His Asn Ala Ser Gly Lys Glu Ala Lys Val Cys Thr Ile Ser Pro Ile Met Gly Tyr Ser Thr Pro Trp Arg Tyr Leu Asp Phe Asn Ala Leu Asn Leu Phe Phe Ser Pro Leu Glu Phe Gln His Leu Ile Glu Asn Tyr Gly Ser Ile Ala Pro Asp Ala Leu Thr Val Thr Ile Ser Glu Ile Ala Val Lys Asp Val Thr Asp Lys Thr Gly Gly Gly Val Gln Val Thr Asp Ser Thr Thr Gly Arg Leu Cys Met Leu Val Asp His Glu Tyr Lys Tyr Pro Tyr Val Leu Gly Gln Gly Gln Asp Thr Leu Ala Pro Glu Leu Pro Ile Trp Val Tyr Phe Pro Pro Gln Tyr Ala Tyr Leu Thr Val Gly Asp Val Asn Thr Gln Gly Ile Ser Gly Asp Ser Lys Lys Leu Ala Ser Glu Glu Ser Ala Phe Tyr Val Leu Glu His Ser Ser Phe Gln Leu Leu Gly Thr Gly Gly Thr Ala Ser Met Ser Tyr Lys Phe Pro Pro Val Pro Pro Glu Asn Leu Glu Gly Cys Ser Gln His Phe Tyr Glu Met Tyr Asn Pro Leu Tyr Gly Ser Ser Arg Val Asp Leu Gln

Fig. 2-10

PAV-1-N

Met Thr Ile Thr Asn Ser Asp His Met Ser Lys Lys Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala Lys Ala Val Tyr Gln Gln Phe Val Glu Phe Tyr Glu Lys Val Thr Gly Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile Ser Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser Ser Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser Glu Asp Leu His Lys Pro Gly Gln Val Ser Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Val Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Ala Ala Pro Val Ala His Phe Gln Gly Ser Leu Pro Glu Val Pro Ala Tyr Asn Ala Ser Glu Lys Tyr Pro Ser Met Thr Ser Val Asn Ser Ala Glu Ala Ser Thr Gly Ala Gly Gly Gly Gly Gly Ser Asn Ser Val Lys Ser Met Trp Ser Glu Gly Ala Thr Phe Ser Ala Asn Ser Val Thr Cys Thr Phe Ser Arg Gln Phe Leu Ile Pro Tyr Asp Pro Glu His His Tyr Lys Val Phe Ser Pro Ala Ala Ser Ser Cys His Asn Ala Ser Gly Lys Glu Ala Lys Val Cys Thr Ile Ser Pro Ile Met Gly Tyr Ser Thr Pro Trp Arg Tyr Leu Asp Phe Asn Ala Leu Asn Leu Phe Phe Ser Pro Leu Glu Phe Gln His Leu Ile Glu Asn Tyr Gly Ser Ile Ala Pro Asp Ala Leu Thr Val Thr Ile Ser Glu Ile Ala Val Lys Asp Val Thr Asp Lys Thr Gly Gly Gly Val Gln Val Thr Asp Ser Thr Thr Gly Arg Leu Cys Ser Asn


VP-1
VP-2
PAN 1
PCE 1
PAN 2
PAN 3
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PAPEP5
PAPEP6
PAPEP7
PAPEP 8

Fig. 3